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Photosynthetic Carbon Reduction and Carbon Oxidation Cycles are the Main Electron Sinks for Photosystem II Activity During a Mild Drought

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Stomatal closure can explain the inhibition of net CO_2 uptake by a leaf subjected to a mild drought: the photosynthetic apparatus appears resistant to lack of water. Changes in both the water content of leaves maintained in a constant environment and the ambient CO_2 molar fraction during measurements on well-hydrated leaves lead to similar effects on net CO_2 uptake and whole chain electron transport as estimated by leaf chlorophyll fluorescence measurements. In particular, it is shown that photosystem II (PSII) functioning and its regulation are not qualitatively changed during desiccation and that the variations in PSII photochemistry can simply be understood by changes in substrate availability in this condition. Moreover, an analysis of the literature shows that when inhibition of net CO_2 uptake by C_3 leaves under drought (*Phaseolus vulgaris* L., *Helianthus annus* L. and *Solanum tuberosum* L.) was lower than 80 %, elevated CO_2 completely restored the photosynthetic capacity. The CO_2 molar fraction in the chloroplasts declines as stomata close in drying leaves. As a consequence, in C_3 plants, ribulose-1,5-bisphosphate oxygenation increases and becomes the main sink for photosynthetic electrons. Depending on the prevailing photon flux density, the O_2 uptake through photorespiratory activity can entirely replace carbon dioxide as an electron acceptor, or not. The rate of the Mehler reaction remains low and unchanged during desiccation. However, drought could also involve CO_2-sensitive modification of the photosynthetic metabolism depending on plant growth conditions and possibly also on plant species. © 2002 Annals of Botany Company

Key words: Chloroplastic carbon dioxide molar ratio, drought stress, high CO_2 effect, *Lavatera trimestris* L. var. *splendens*, *Pisum sativum* L., photorespiration, photosynthesis, stomatal effect.

INTRODUCTION

Reductants produced by linear electron transfer in the thylakoid membrane are mainly used to reduce carbon dioxide and dioxygen in the chloroplasts. In C_3 plants this occurs mainly through the activity of ribulose 1,5-bisphosphate carboxylase/oxygenase (Rubisco) (Ruuska *et al.*, 2000), which is the first enzyme of both the photosynthetic carbon reduction (PCR) and carbon oxidation (PCO) cycles. For most C_4 plants at ambient CO_2 molar ratio (C_a), as Rubisco oxygenase is inhibited by the high CO_2 molar ratio in the bundle sheath cells, oxygenation of ribulose bisphosphate (RuBP) (if any) is low, and dioxygen reduction occurs mainly through both the Mehler reaction and the activity of Cytochrome a3 and the alternative oxidase in the mitochondrion. CO_2 and O_2 are competitors at the catalytic sites of Rubisco and the nature of this competition is well documented and modelled (Von Caemmerer, 2000). The reactions that are responsible for direct reduction of O_2 have been reviewed recently (Asada, 1999; Foyer and Noctor, 2000). They mainly include mechanisms at the photosystem I (PSI) level, either reduced ferredoxin or FeS-X centres, although plastoquinone and P680 in its triplet state can also reduce O_2.

The electrons produced by photosystem II (PSII) activity are also used to reduce acceptors other than CO_2 and O_2. In particular, nitrogen assimilation by photosynthetic cells may require 10–20 % of the total electron flow (Champigny, 1995). Oxaloacetate (OAA) can also be reduced to malate by NADPH when the activation state of $NADP^+$-malate dehydrogenase is high enough. As this activation state is driven by the reduction of disulfur bonds on the enzyme, this reaction is considered to be a valve which allows a flow of reducing equivalents to the cytoplasm and the mitochondrion through the OAA/malate shuttle, and thus is a redox state control of the stroma (Scheibe, 1987). Some of the electrons reducing ferredoxin can also be recycled to the plastoquinone pool (Arnon and Chain, 1975). Direct O_2 reduction through the Mehler reaction, cyclic electron flow and functioning of the malate valve are only linked with the build-up of a low pH in the lumen and thus contribute to maintaining both ATP/NADPH at a value required for net CO_2 assimilation and a trans-thylakoidal ΔpH large enough to regulate the photosynthetic photochemistry and electron transfer in a fluctuating environment.

Fluxes of electrons in the photosynthetic membrane are highly regulated and depend strongly on both substrate availability and the redox state of the transfer paths. In C_3

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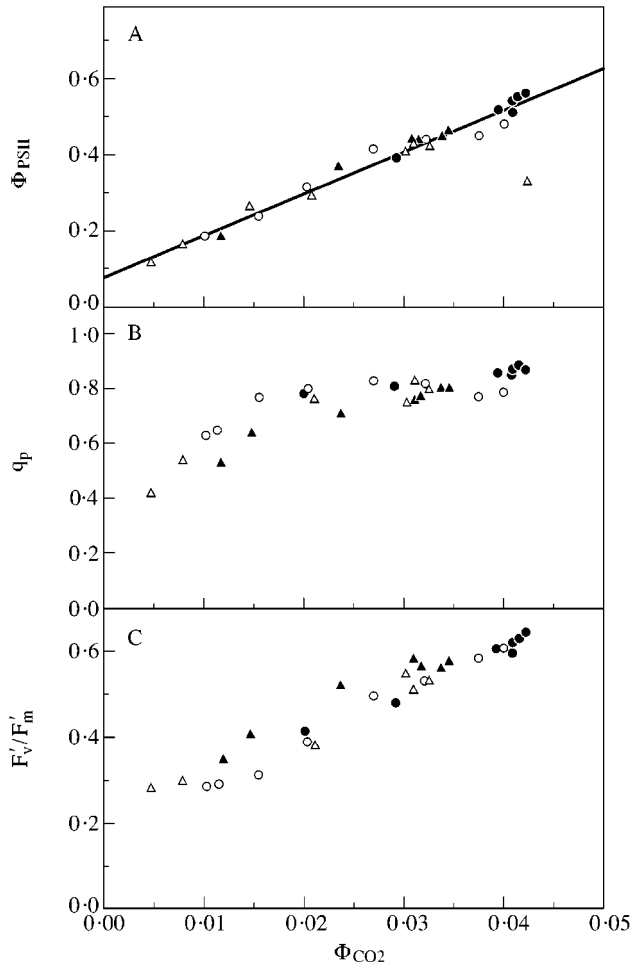

FIG. 1. Φ_{PSII} (A), q_p (B) and F_v'/F_m' (C) as a function of Φ_{CO2}. Measurements were made on *Zea mays* L. plants either at different ambient CO_2 molar ratios (C_a) on well-hydrated leaves (closed circles) or after water was withheld, on dehydrating leaves (open circles) at normal C_a (about 360 ppm). Gas exchange chlorophyll fluorescence emission measurements were coupled as described in Cornic and Briantais (1991). Conditions during measurements were as follows: leaf temperature, 23·5 ± 0·6 °C; O_2 molar ratio, 21 % in nitrogen; vapour pressure deficit (VPD), 0·7 ± 0·1 kPa; photon flux density (PFD), 450 µmol m^{-2} s^{-1} (K. Saccardy and G. Cornic, unpubl. res.).

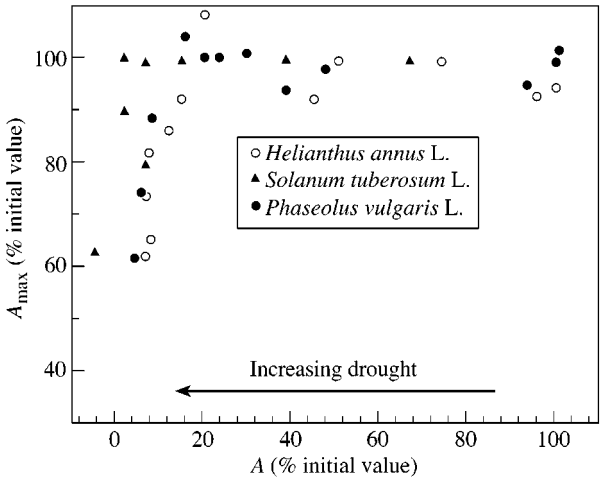

FIG. 2. Relationship between A_{max}, the net photosynthesis (net CO_2 or net O_2 evolution) measured at elevated C_a (up to 10 %) and A, the net CO_2 uptake by leaves maintained at normal C_a. Both A_{max} and A were measured during a drought. A is taken as an indicator of the water status in the leaf: it decreases as desiccation increases. Data are taken from Cornic *et al.* (1989, Figs 1 and 2A), Graan and Boyer (1990, Fig. 5A) and Tourneux and Peltier (1995, Figs 4C and 5).

plants particularly, decreasing the ambient CO_2 molar fraction under saturating light (Rubisco-limited situation) causes a decline in the whole chain electron transport rate, while under limiting light (electron-limited situation) whole chain electron transport remains unchanged even at very low C_a (Badger, 1985; Cornic and Briantais, 1991).

The extent to which drought affects the rate of electron transfer in the thylakoid membranes and the partitioning of the electrons produced by PSII activity between the potential acceptors in the chloroplasts has been a matter of debate in the past decade. A discussion of electron transfer during drought must first include both an assessment of the resistance of the photosynthetic system to drought, and an understanding of the substrates available to the chloroplasts in this condition. Here we summarize evidence showing that the photosynthetic apparatus is resistant to drought, and that when stomata close in dehydrating plants, the electrons produced by PSII are mainly used to drive the oxygenation of RuBP. We also show that CO_2-sensitive photosynthetic reactions are inhibited in pea plants growing at low temperature and subjected to drought.

THE PHOTOSYNTHETIC APPARATUS IS RESISTANT TO DROUGHT

It has often been shown that the maximum quantum yield of PSII photochemistry measured by the ratio F_v/F_m (the variable to the maximum chlorophyll fluorescence emissions) after a long period of dark adaptation is not changed even by a severe drought (relative water content of 50 to 40 %; e.g. Cornic *et al.*, 1987; Genty *et al.*, 1987). Likewise the maximum quantum yield of PSI measured at the temperature of liquid nitrogen is not affected in this condition (Cornic *et al.*, 1987). As a whole, linear electron transfer is very resistant to drought (Kaiser *et al.*, 1981; Sharkey and Badger, 1982; Genty *et al.*, 1987) and can remain unchanged in isolated *Xanthium strumarium* mesophyll cells (Sharkey and Badger, 1982) down to a water potential of –4·0 MPa, showing an inhibition of only about 20 % at –6·4 MPa.

Figure 1 shows variation in Φ_{PSII} (the operating quantum yield of PSII photochemistry), q_p (a relative estimation of open PSII centres) and F_v'/F_m' (the quantum yield of open PSII centres) as a function of Φ_{CO2} (the quantum yield of gross CO_2 uptake) measured on *Zea mays* L. leaves in normal air (21 % $O_2 + N_2$). As shown by Genty *et al.* (1989) these three parameters are related as followed:

$$\Phi_{PSII} = q_p(F_v'/F_m')$$

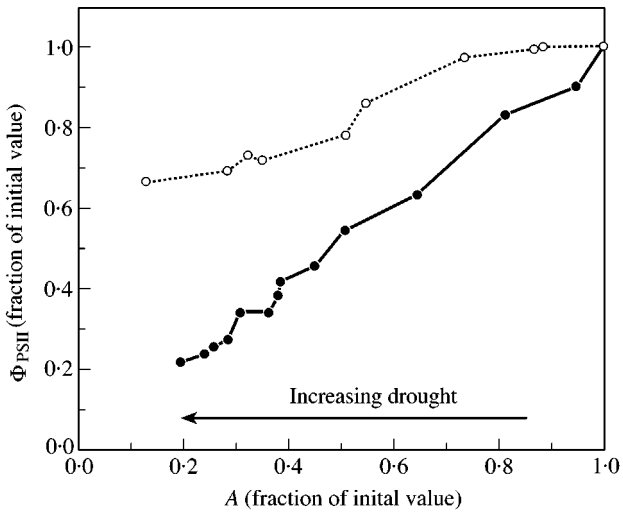

FIG. 3. Relationships between relative value of Φ_{PSII} and *A* during desiccation of detached leaves from *P. vulgaris*. The O_2 molar fraction during measurements was either 21 % (closed circles) or 1 % (open circles) in nitrogen. Other conditions were as follows: leaf temperature, 23·5 °C initially, increasing to 24·9 °C at the end of the measurements; PFD, 450 μmol m^{-2} s^{-1}; and C_a, about 360 ppm. Relative water content of the leaves at the end of the experiments was 76·2 and 72·8 % in 21 and 1 % O_2, respectively (G. Cornic, unpubl. res.).

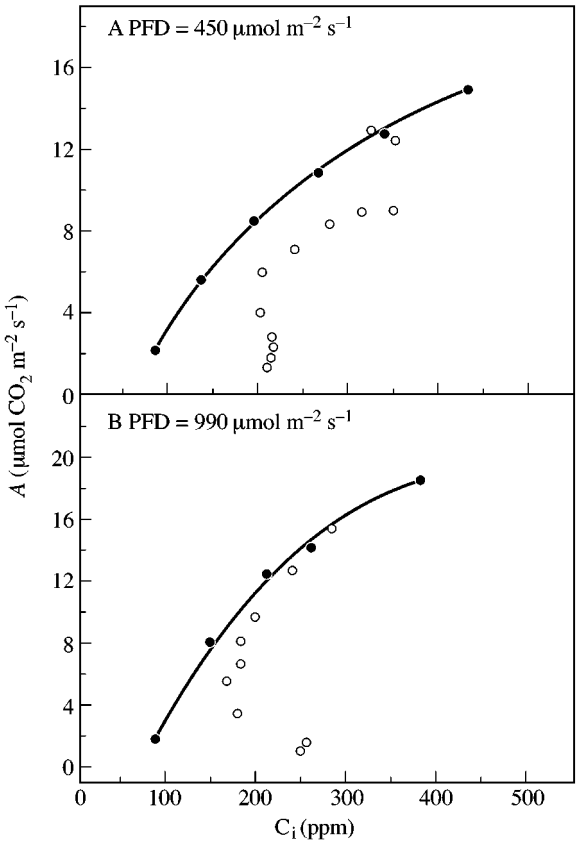

FIG. 4. Relationship between *A* and the leaf internal CO_2 molar ratio (C_i) measured on detached *Lavatera trimestris* L. leaves either before (well-hydrated, closed circles) or after (open circles) a rapid desiccation. Plants were grown in winter, in a peat soil, in a temperature-controlled glasshouse in the University of Illinois at Urbana-Champaign, USA (temperature from about 20 °C during the day to 17 °C during the night). Measurements were made with a LICOR 6400 under either a PFD of 450 (A) or 990 (B) μmol m^{-2} s^{-1}. Other conditions were as follows: leaf temperature 25 ± 0·3 °C; C_a, 350 ppm; and VPD, 1·5 ± 0·5 kPa. Relative water content of the leaves at the end of the experiments was 70·4 and 67·7 % for A and B, respectively (G. Cornic and S. Long, unpubl. res.).

Measurements were made on leaves of plants growing in a growth cabinet and either watered regularly or allowed to dehydrate slowly. In both cases the relationships were the same, showing clearly that drought did not change PSII functioning or the regulation of its functioning. In agreement with Cornic (1994), measurements also showed that drought did not induce the build-up of an extra electron sink.

In *Z. mays* leaves, the rate of electron transfer to sinks other than CO_2 was low (see the intercept with the vertical axis of the relation between Φ_{PSII} and Φ_{CO2}). Similar results were obtained with C_3 plants in nitrogen with 1 % O_2 to inhibit photorespiration (see Cornic, 1994). Provided the relationship between Φ_{PSII} and Φ_{CO2} (as in Fig. 1) is the same for well-watered and drying plants, it is possible to use Φ_{PSII} to calculate the rate of whole chain electron transport during drought, assuming that the way the electrons are used does not change the quantum yield of PSII photochemistry. In this case Φ_{PSII} can also be used directly as an indicator of the relative variation of whole chain electron transfer if the drying plants are under constant light.

In addition, measurements performed at very high C_a show that the mechanisms that are responsible for the maximum rate of photosynthesis, including ATP synthesis, are resistant to drought (Fig. 2). However, data from the literature are not easy to compare as the variations *A* (net CO_2 assimilation by leaves) measured at normal C_a (about 350 ppm) and A_{max} measured at elevated C_a (up to 10 %) during drought are plotted either as a function of leaf relative water deficit or leaf water potential. A comparison between these two types of data becomes possible if *A* itself is taken as an indicator of the desiccation state of the leaves: a decrease in *A* indicating an increase in desiccation. Figure 2 does indeed show that elevated C_a could restore the full capacity of photosynthesis (A_{max}) of *P. vulgaris* (Cornic *et al*., 1989) and of *H. annus* (Graan and Boyer, 1990) until *A* measured at normal C_a was inhibited by 80 % by desiccation. An inhibition of *A* by 80 % corresponds to a relative leaf water deficit of about 25 % and to a leaf water potential of –1·2 MPa for *P. vulgaris* and *H. annuus*, respectively. The range of leaf water deficit over which A_{max} remains changed, or changes only little, although *A* declines, could characterize what is termed in this paper 'a mild drought'. Results obtained by Tourneux and Peltier (1995) on *Solanum tuberosum* are also plotted (Fig. 2). They show a very similar relationship although they were obtained by measuring $^{16}O_2$ and $^{18}O_2$ using mass spectrometry. It is worth noting that in the three experiments described in Fig. 2, drought was induced either by withholding the watering of whole plants (desiccation occurring in a couple of weeks in *P. vulgaris* or in 2–3 d in *H. annuus*) or by drying in the dark (*S. tuberosum* leaf discs). Kaiser (1987) also obtained very similar data.

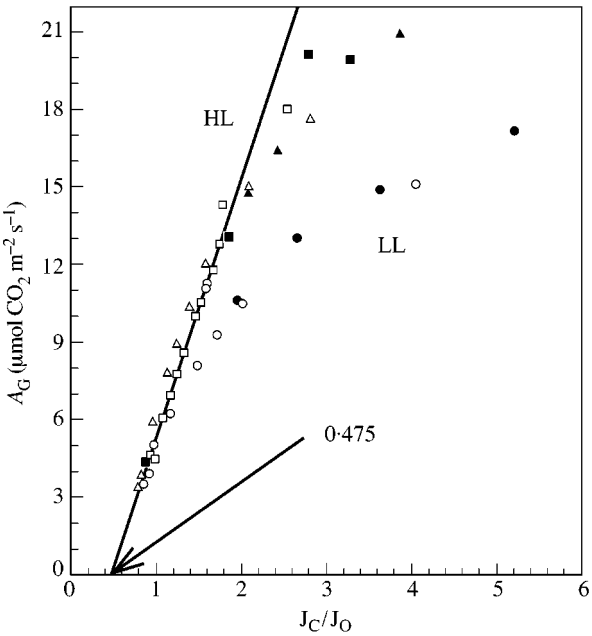

FIG. 5. Relation between A_G (estimation of gross photosynthesis = A + dark respiration) and J_c/J_o where J_c is the rate of RuBP carboxylation and J_o the rate of RuBP oxygenation calculated as in Epron and Cornic (1993) by measuring chlorophyll fluorescence emission (with an FMS2, Hansatech fluorometer) in parallel with net CO_2 uptake during the experiments presented in Fig. 4. As explained in the text, the ratio J_c/J_o is proportional to C_c, the carbon dioxide molar fraction in the chloroplasts. Measurements were performed at a PFD of 450 µmol m^{-2} s^{-1} (LL, circles) or under a PFD of 990 µmol m^{-2} s^{-1} (HL, triangles and squares), on well-hydrated leaves (closed symbols) at different C_a, or during dehydration of the cut leaves at constant C_a (open symbols). Data were taken from Fig. 4A and B (circles and triangles). Other data from measurements taken under a PFD of 990 µmol m^{-2} s^{-1} were added (squares). Experimental conditions as in Fig. 4. The correlation line was calculated for all data corresponding to J_c/J_o < 1·4 (G. Cornic and S. Long, unpubl. res.).

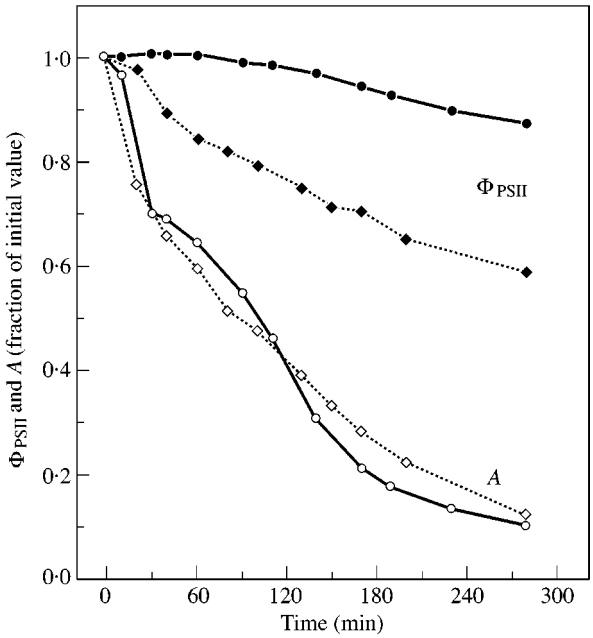

FIG. 6. Variation in relative values of Φ_{PSII} and A during desiccation of cut leaves of *L. trimestris*. Chlorophyll fluorescence emission was done in parallel with net CO_2 uptake during the experiments (see Figs 4 and 5). Closed symbols, Φ_{PSII} measurements; open symbols, A measurements. Light conditions 450 (circles) or 990 (squares) µmol m^{-2} s^{-1}. These measurements were performed simultaneously with the measurements presented in Fig. 4A and B.

IN C_3 PLANTS, ELECTRONS PRODUCED BY PSII ACTIVITY ARE MAINLY USED TO RUN THE PCR AND PCO CYCLE

Estimation of whole chain electron transfer on intact leaves can be done either by using chlorophyll fluorescence emission measurements as explained above, or by measuring the net O_2 CO_2-dependent evolution together with $^{18}O_2$ uptake using a mass spectrometer. In this case, addition of the two values gives gross photosynthesis which is proportional to the whole chain electron transfer rate. Measurements performed to date by either of these methods on C_3 leaves subjected to drought indicate that electrons are produced in excess of the amount needed for CO_2 assimilation (e.g. Cornic and Briantais, 1991; Tourneux and Peltier, 1995; Biehler and Fock, 1996). Thomas and André (1982) were the first to note that there was a relative increase in O_2 uptake while A was declining in soybean subjected to drought stress. This was largely confirmed by others. As an example, Fig. 3 shows the relative variation in Φ_{PSII} as a function of the relative values of A measured in air containing either 21 or 1 % O_2 and normal C_a (350 ppm) on dehydrating cut bean leaves. When A has decreased to 20 % of its initial value, Φ_{PSII} is inhibited by more than 80 % in 1 % O_2, while it has declined only by about 35 % in 21 % O_2. This shows that oxygen can indeed efficiently replace carbon dioxide as an electron acceptor. The small decline in Φ_{PSII} during the desiccation of the leaf in 21 % O_2 could be due to a limitation of ribulose 5-phosphate regeneration in this condition (see discussion of Fig. 6).

Given the *in vitro* specificity factor of Rubisco, and assuming that $^{18}O_2$ uptake was mainly due to photorespiratory activity, Tourneux and Peltier (1995) were able to use gas exchange data to calculate values for chloroplast CO_2 molar fraction (C_c) in dehydrating potato leaves that agreed closely with published values obtained by $^{13}C/^{12}C$ isotope discrimination. This suggests that RuBP oxygenation is indeed the main path for O_2 uptake. Moreover, it is possible to calculate a specificity factor for Rubisco using gas exchange and chlorophyll fluorescence emission data for *P. vulgaris* leaves that agrees well with published values (Cornic and Briantais, 1991; Ghashghaie and Cornic, 1994), showing that alternative electron fluxes, including the Mehler reaction, are not quantitatively important. This is also consistent with Fig. 1, which shows that drought did not induce increased alternative electron fluxes on slowly dehydrating *Z. mays* leaves. Using transgenic tobacco plants with reduced Rubisco activity and a large electron transport

capacity relative to Rubisco oxygenase, Ruuska *et al*. (2000) came to the same conclusion, both at high C_a and at the CO_2 compensation point which is supposed to mimic a drought-stressed condition. Consistently, Badger *et al*. (2000) could not see any increase in the Mehler reaction in dehydrating Madrone (*Azbutus menziesii*) leaves.

As noted by Noctor and Foyer (1998), in the absence of ATP consumption, the Mehler reaction might not occur because of an efficient photosynthetic control in this condition. In a leaf under water stress and having a low photosynthetic rate, ATP consumption is likely to be low.

NET CO_2 UPTAKE BY LEAVES SUBJECTED TO DESICCATION DECLINES BECAUSE STOMATAL CLOSURE REDUCES CO_2 AVAILABILITY TO THE CHLOROPLASTS

The above discussion provides a model that can be tested: if the proportion of photosynthetic electrons allocated to the reduction of O_2 increases in the chloroplasts during drought because of photorespiratory activity, then we expect the CO_2 molar fraction in the leaf to decrease at the same time (Fig. 3).

Figure 4 shows the relationship between A and C_i (the CO_2 molar fraction at the evaporating sites within the leaf) obtained first by changing C_a around a *Lavatera trimestris* L. (var. *splendens*) leaf and secondly by dehydrating the same leaf rapidly (within 4 h) at normal C_a. The experiment was done both under limiting and nearly saturating light. Although there was a clear decline in C_i when the leaves began to wilt, the A/C_i curves during dehydration were not the same as the A/C_i curves measured on well-hydrated leaves. The differences between the two were particularly obvious for measurements done under low light (Fig. 4A) and clearly evident when C_i was lower than 200 ppm for measurements done under high light (Fig. 4B). The differences can be attributed to both patchy stomatal closure (which might have been important because of the rapid dehydration of the leaves) and, when the fluxes are low, to the increasing importance of cuticular transpiration (Boyer *et al*., 1997). However, what it is really important to know is the CO_2 molar fraction at the chloroplast level, C_c, or else a relative value of C_c.

Knowing the relation between Φ_{PSII} and Φ_{CO2} in a non-photorespiratory condition for *L trimestris* leaves, and using parallel measurements of Φ_{PSII} during the two sets of measurements described in Fig. 4, it is possible to calculate the rate of carboxylation (J_c) and the rate of oxygenation (J_o) by the leaves (Epron and Cornic, 1993). As the oxygen uptake in this situation is mainly due to oxygenase activity (see the discussion in the preceding section), J_c/J_o is related to C_c as follows:

$$J_c/J_o = S(C_c/O)$$

where O is the concentration of oxygen dissolved in the chloroplasts and S the O_2/CO_2 specificity factor of Rubisco.

As the O concentration is not expected to change much during these measurements, this relation shows that plotting

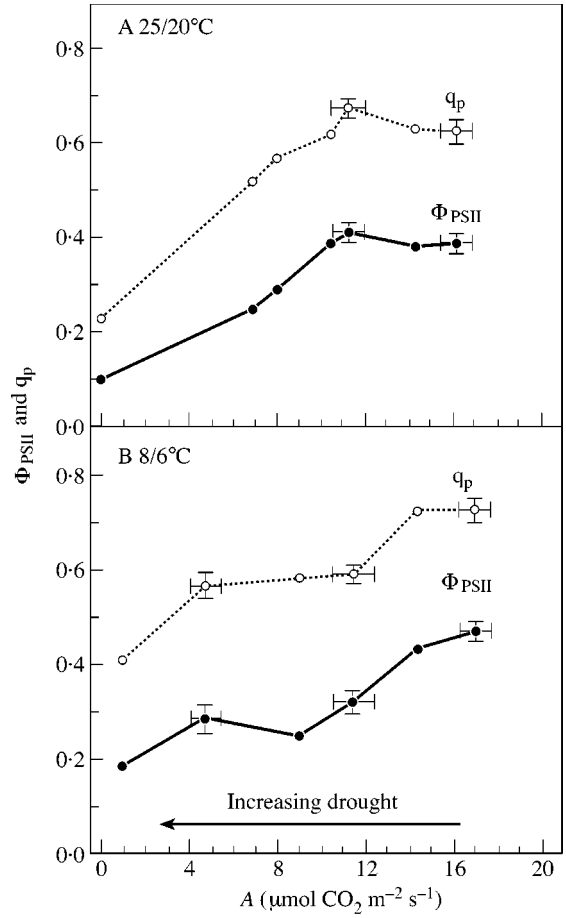

FIG. 7. Variation in Φ_{PSII} and q_p as a function of A during dehydration of *Pisum sativum* L. plants obtained by withholding water. Plants were grown on a peat soil in a growth cabinet and watered every 2 d. A, Plants grown at 25/20 °C (day/night), 16 h light period (normal temperature); relative water content of the leaves was about 62 % at the end of the experiment. B, Plants grown at 8–9/6 °C (day/night), 16 h light period (low temperature); relative water content of the leaves was about 69 % at the end of the experiment. In both conditions, PFD at the top of the plants during growth was 350 μmol m^{-2} s^{-1}. Measurements were made as explained in Fig. 1. Conditions during measurements were: leaf temperature, 23·5 ± 0·5 °C; C_a, 360 ± 5 ppm; VPD, 0·6 ± 0.2 kPa; and PFD, 450 μmol m^{-2} s^{-1}. The standard deviation of the mean is shown when it was possible to calculate the mean of three to five close measurements (N. Manuel and G. Cornic, unpubl. res.).

A as a function of J_c/J_o is equivalent to plotting A as a function of C_c if S remains constant.

Figure 5 shows that the relationship between A_G (estimation of gross photosynthesis: = A + dark respiration) and J_c/J_o is the same when A is measured at different C_a or at the same C_a when the leaf is dehydrating (data from Fig. 4 and additional measurements). This observation strongly suggests that: (1) net CO_2 uptake during dehydration declines only because the CO_2 molar fraction is declining in the chloroplasts; and (2) S is not changed during desiccation. It is worth noting that the value of J_c/J_o shown in Fig. 5 is what we expect at the different C_i assuming a specificity factor of 80, which is commonly

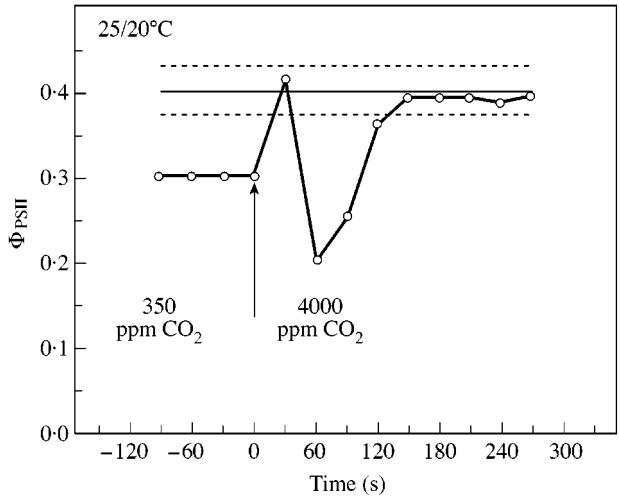

FIG. 8. Typical variation over time of Φ_{PSII} when C_a was changed abruptly from 350 to 4000 ppm around dehydrated leaves of *P. sativum* plants grown at normal temperature (relative water content of the leaf = 68·2 %). Other experimental conditions were as in Fig. 7. The line parallel to the *x*-axis is the mean Φ_{PSII} value measured on well-hydrated leaves ± s.e. (dotted lines, n = 4).

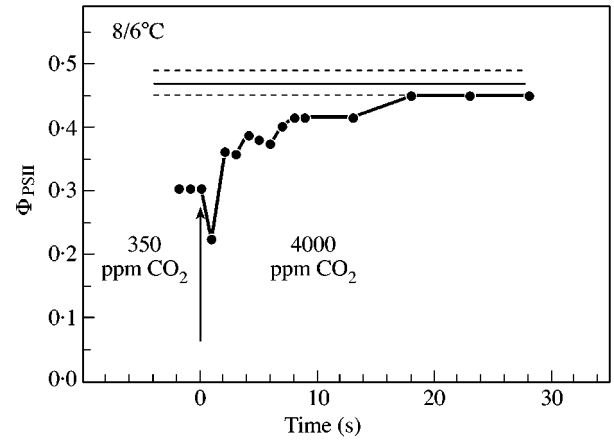

FIG. 9. Typical variation over time of Φ_{PSII} when C_a was changed abruptly from 350 to 4000 ppm around dehydrated leaves of *P. sativum* plants grown at low temperature (relative water content of the leaf = 74·5 %). For details see Fig. 8 legend.

measured in C_3 plants, and that the J_c/J_o ratio at Γ_* (when $A_G = 0$) is very close to the theoretical value of 0·5.

Using these data, it is also possible to calculate the actual C_c values assuming that $\Gamma_* = 42{\cdot}5$ ppm at a leaf temperature of 25 °C (Brooks and Farquhar, 1985; Bernacchi *et al.*, 2001) and considering the relationship:

$$C_c = 2\Gamma_* (J_c/J_o)$$

C_c values for leaves maintained under high light at normal C_a are about 50 ppm lower than C_i, and the ratio of C_c/C_a is 0·47 and 0·57 when C_a = 340 ppm, depending on the assumption about dark respiration when calculating J_c and J_o. These values are in good agreement with published values (for a discussion see Tourneux and Peltier, 1995).

During dehydration of *L. trimestris* leaves (Figs 4 and 5), *A* declines in a similar fashion in high and low light (Fig. 6), and is less than 20 % of its initial value after 5 h of dehydration. However, Φ_{PSII} decreased by only 10 and 40 % under low and high light, respectively. It is worth noting that under low light after 2 h of dehydration, Φ_{PSII} had hardly changed whereas *A* was already inhibited by about 60 %. This is to be expected if *A* is limited by C_c during desiccation: under limiting light (electron-limiting situation) the electron transfer rate does not decline, but it does under high light (Rubisco-limiting situation) (Badger, 1985). A similar observation was made by Biehler and Fock (1996) who measured whole chain electron transport (measurement of $^{16}O_2$ and $^{18}O_2$ by mass spectrometry) both under saturating and limiting light, together with net CO_2 uptake, by slowly dehydrating leaves of *Triticum* plants. Thus we expect an absolute increase in photorespiration rate in C_3 leaves dehydrating under limiting light and only a relative (compared with net CO_2 uptake) increase when C_3 leaves are dehydrating under saturating light.

DROUGHT CAN ALSO INDUCE INHIBITION OF PHOTOSYNTHETIC METABOLISM WHICH IS SENSITIVE TO CO_2

Figure 7 shows the relationship between Φ_{PSII} and *A* measured at a leaf temperature of 23·5 ± 0·6 °C during slow dehydration (10–15 d after water was withheld) of *Pisum sativum* L. plants grown either at normal (25/20 °C, day/night) or low temperature (8–9/6 °C, day/night). Φ_{PSII} of plants grown at normal temperature remained constant while net CO_2 uptake by dehydrating leaves decreased by about 35 %. In contrast, Φ_{PSII} declined as soon as *A* was inhibited by drought in plants grown at low temperature. In both growth conditions the relation between q_p and *A* during drought was similar to the relationship between Φ_{PSII} and *A*. In plants grown at normal temperature, there was clearly another electron acceptor during the early stage of the drought (presumably O_2; see Fig. 3) which entirely replaced CO_2.

To check whether the decline in Φ_{PSII} observed during drought was due to an inhibition of whole chain electron transport by low C_c or by damage to photosynthetic processes caused by the decreasing water content of the leaves, we abruptly increased C_a from 350 to 4000 ppm around wilted leaves. For plants grown at normal temperature (Fig. 8), such a shift in C_a values caused an immediate increase in Φ_{PSII}, in an oscillating mode, from 0·3 to the control value, showing that whole chain electron transfer was limited only by the availability of carbon dioxide. The effect of drought on Φ_{PSII} of leaves of plants grown at low temperature was also removed when C_a was increased to 4000 ppm. However, the increase from 0·3 to the control value was much slower and, as shown in Fig. 9, was only complete after about 20 min. In plants grown at low temperature, it is likely that an inhibition of CO_2-sensitive photosynthetic reaction(s) was induced during drought. Comparing ABA-treated with drought-stressed leaves, Graan and Boyer (1990) noted that 'changes' other than stomatal closure occur 'at low leaf water potential that are

not present in ABA-treated leaves'. These authors concluded 'the nature of these changes is not known but appears to involve an altered but CO_2-sensitive photosynthetic metabolism'. Inactivation of Rubisco by low C_i in wilted leaves might be part of these CO_2-sensitive processes, as suggested by Meyer and Genty (2000). However, because ABA-treated leaves, in which C_i is expected to be low, did not behave like wilted leaves (Graan and Boyer, 1990) and because high CO_2 slowly removed the inhibition of Φ_{PSII} in dehydrated leaves of pea plants grown at low temperature (Fig. 9), all the CO_2-sensitive inhibition cannot be due to desiccation. Sucrose Phosphate Synthase (SPS) activity is generally inhibited during drought (Vassey and Sharkey, 1989; Vassey *et al*., 1991; Castrillo, 1992), due presumably to the low carbon dioxide molar fraction prevailing in the leaf when stomata close. Moreover this inhibition can be largely removed at elevated C_a (Vassey *et al*., 1991). Such a decline in SPS activity in dehydrating leaves could lead to a P_i limitation of photosynthesis. We can thus hypothesize that exportation of triose phosphates is involved in the CO_2-sensitive process inhibited by drought. Presumably, this effect could depend both on growth conditions, as shown here by the comparison between *P. sativum* plants grown at normal and low temperature, and on the plant species.

CONCLUSIONS

Up to a leaf relative water content of about 70 %, it is likely that stomatal closure plays the main role in the decline in leaf photosynthesis and that the photosynthetic machinery remains intact. This allows the leaf to respond rapidly to changes in the water status of the environment. As shown here in Fig. 2, the lower limit of this 'safe' range might be given by the leaf relative water content (or the leaf water potential) causing about 80 % of inhibition of net CO_2 uptake by the leaf under drought. Under such circumstances, oxygenation of RuBP in C_3 plants can efficiently replace carboxylation of RuBP when the stomata close. Nevertheless, in this situation the activity of whole chain electron transport can be down-regulated if the electron flow is sink-limited, as is likely to occur when the leaf is exposed to saturating or nearly saturating light conditions. This will of course cause a concomitant increase in non-radiative dissipation of the energy trapped at the PSII level as discussed by Weis *et al*. (1987) and Genty *et al*. (1989). As shown here in Fig. 1 and detailed in Cornic (1994), drought does not qualitatively change the regulation of PSII activity. However, depending on growth conditions and on the plant species, drought could inhibit some parts of the photosynthetic metabolism. As shown in Fig. 9, this inhibition is removed by high CO_2. This type of response to stomatal closure (Cornic, 1994) implies that some reactions of photosynthetic metabolism are down-regulated, presumably due to low CO_2.

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